

**WHAT IS CLAIMED IS:**

1. A construct comprising a homogeneous conjugate of formula A-L-P,  
wherein

5 A represents a hepatic ligand that specifically binds to a hepatic receptor, thereby facilitating the entrance of said conjugate into cells having said receptor;

10 L represents a bifunctional linker that is covalently linked to A in a regiospecific manner to form A-L; A-L is covalently linked to P in a regiospecific manner to form A-L-P;

15 P represents a biologically stable oligomer, wherein P is released from the conjugate following hydrolysis or reduction of at least one specific biochemical linkage, and contains internucleotide linkages resistant to enzymatic hydrolysis or biodegradation upon release from the conjugate.

2. The construct of claim 1, wherein said oligomer is an oligonucleotide, an oligonucleotide analog or an oligonucleoside.

3. The construct of claim 1, wherein said oligomer binds to a hepatic pathogen.

4. The construct of claim 3, wherein said pathogen is a hepatic virus.

5. The construct of claim 3, wherein said pathogen is a liver parasite.

6. The construct of claim 4, wherein said virus is a hepatitis virus.

7. The construct of claim 6, wherein said hepatitis virus is hepatitis B virus.

8. The construct of claim 7, wherein said oligomer binds to a surface antigen of said virus.

9. The construct of claim 7, wherein said oligomer binds to a core antigen of said virus.

10. The construct of claim 7, wherein said oligomer binds to an encapsidation sequence of said virus.

11. The construct of claim 6, wherein said hepatitis virus is a hepatitis C virus.

12. The construct of claim 6, wherein said hepatitis virus is a hepatitis D virus.

13. The construct of claim 5, wherein said parasite is plasmodium for malaria.

20 14. The construct of claim 8, wherein said surface antigen is an S-gene antigen.

15. The construct of claim 9, wherein said core antigen is a C-gene antigen.

25 16. The construct of claim 7, wherein said oligomer binds to an RNA preS1 open reading frame sequence.

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17. The construct of claim 6 comprising a sequence selected from the group consisting of GTTCTCCATGTCAG, TTTATAAGGGTCGATGTCCAT, and AAAGCCACCCAAGGCA.

5 18. The construct of claim 2, wherein said oligomer further comprises deoxyribose methylphosphonate internucleotide linkages.

19. The construct of claim 2, wherein said oligomer comprises deoxyribose phosphorothioate internucleotide linkages.

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20. The construct of claim 2, wherein said oligomer comprises phosphodiester linkages.

21. The construct of claim 2, wherein said oligomer comprises a  
15 combination of deoxyribose methylphosphonate/phosphorothioate internucleotide linkages.

22. The construct of claim 2, wherein said oligomer further comprises a  
15 combination of deoxyribose methylphosphonate/phosphodiester  
20 internucleotide linkages.

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23. The construct of claim 2, wherein said oligomer comprises  
deoxyribose phosphorothioate/phosphodiester internucleotide linkages.

25 24. The construct of claim 2, wherein said oligomer comprises 2'-O-  
methylribose methylphosphonate internucleotide linkages.

25. The construct of claim 2, wherein said oligomer comprises 2'-O-  
methylribose phosphorothioate internucleotide linkages.

26. The construct of claim 2, wherein said oligomer comprises 2'-O-methylribose phosphodiester internucleotide linkages.

5 27. The construct of claim 2, wherein said oligomer comprises a combination of 2'-O-methylribose methylphosphonate/2'-O-methylribose phosphodiester internucleotide linkages.

10 28. The construct of claim 2, wherein said oligomer comprises a combination of 2'-O-methylribose methylphosphonate/2'-O-methylribose phosphorothioate internucleotide linkages.

15 29. The construct of claim 2, wherein said oligomer comprises a combination of 2'-O-methylribose phosphorothioate/2'-O-methylribose phosphodiester internucleotide linkages.

~~30. A purified ligand-linker construct comprising a liver ligand covalently linked to a bifunctional linker to form the A-L construct.~~

20 31. The purified ligand-linker construct of claim 30, wherein the liver ligand binds specifically to a liver receptor.

32. The purified ligand-linker construct of claim 30, wherein the liver ligand is selected from Figure 1.

25 33. The purified ligand-linker construct of claim 32, wherein the liver ligand is YEE(ah-GalNAc)<sub>3</sub>.

34. The purified ligand-linker construct of claim 30, wherein the bifunctional linker is selected from Table 3 or Table 4.

35. The purified ligand-linker conjugate of claim 34, wherein said 5 bifunctional linker is SMCC.

36. The purified ligand-linker construct of claim 30, wherein said ligand is YEE(ah-GalNAc)<sub>3</sub> and said bifunctional linker is SMCC, and they are conjugated to form the YEB(ah-GalNAc)<sub>3</sub> - SMCC construct.

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~~37.~~ A method for synthesizing conjugates comprising a Conjugation Method 1, wherein

15 a) a 2'-O-Me-nucleotide phosphodiester linkage is incorporated to the 5'-end of the oligonucleotide or oligonucleotide analogs;

b) the 5'-end of the oligonucleotide or oligonucleotide analog is enzymatically phosphorylated using PNK and ATP;

c) the 5'-phosphate group of the oligonucleotide or oligonucleotide analog is modified to introduce a disulfide linkage to form 5'-disulfide-modified oligonucleotide or oligonucleotide analog;

20 d) the 5'-disulfide group of the 5'-disulfide-modified oligonucleotide or oligonucleotide analog is reduced to a thiol group to form a thiol-modified oligonucleotide; and

f) one reactive group of the heterobifunctional linker is covalently conjugated to a ligand and a second group of the heterobifunctional linker is covalently conjugated to said thiol-modified oligonucleotide or oligonucleotide analogs to form the A-L-P conjugate.

38. A method for synthesizing conjugates comprising a Conjugation Method 2 wherein

- a) a ligand is modified with a bifunctional linker to form an A-L construct;
- 5 b) said A-L construct is purified to greater than 95% homogeneity and unreacted linker is removed;
- c) the oligonucleotide or oligonucleotide analog is modified to form a thiol-modified oligomer;
- d) said thiol-modified oligomer is purified under degassed conditions
- 10 to remove unreacted reagent and impurities;
- e) a conjugation reaction using a purified A-L construct and a purified thiol-oligomer in a two-component conjugation reaction is executed under degassed conditions; wherein said conjugation can be performed by using either excess amounts of said ligand scaffold or said thiol-modified oligomer to form purified A-L-P conjugates; and the A-L-P
- 15 conjugate is purified.

39. The method of claim 38, wherein said A-L-P conjugate is purified by size exclusion chromatography.

20 40. The method of claim 39, wherein said size exclusion chromatography is a G-25 column.

41. The method of claim 38, wherein said A-L-P conjugate is purified by

25 using high pressure liquid chromatography.

42. The method of claim 41, wherein said HPLC is reverse phase.

43. The method of claim 38 wherein said ligand binds selectively to a targeted receptor.

44. The method of claim 43, wherein said ligand is selected from the group consisting of an organ-specific ligand.

45. The method of claim 44, wherein said ligand is selected from the group consisting of a liver, lung, kidney, pancreas, breast, prostate, ovarian, and brain.

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46. The method of claim 43, wherein said ligand further comprises a cell-specific ligand.

47. The method of claim 46, wherein said cell-specific ligand further comprises a lymphocyte, macrophage, an epithelial cell, dendritic cell, mast cell, or a granulocyte.

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48. A method for radiolabeling an oligonucleotide-containing or oligonucleotide analog-containing conjugate, comprising radiolabeling an A-LP conjugate, wherein

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a) a tri-nucleotide tracer unit, 5'-T-3'-ps-3'-T-ps-T-5' is added to the 3'-end of an oligonucleotide or an oligonucleotide analog during solid-phase synthesis;

b) said tracer unit undergoes enzymatic phosphorylation using PNK and ATP to form a modified tracer unit; and

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c) said modified tracer unit is chemically modified with an amine of the radioactive phosphate group of the A-L-P conjugate to prevent cellular enzymatic degradation.

49. The method of claim 48, wherein the tracer-containing oligomers are used to synthesize an A-L-P conjugate.

50. The method of claim 48, wherein said amine is a primary amine.

51. The method of claim 50, wherein said primary amine is ethylenediamine.

52. A method for the synthesis of oligonucleotide-containing conjugates wherein

10 a) a bifunctional linker terminating in a disulfide moiety is incorporated onto an oligonucleotide or an oligonucleotide analog during solid-phase synthesis to form a disulfide-modified oligomer;

15 b) said disulfide-modified oligomer is purified;

c) the disulfide moiety of said disulfide-modified oligomer is reduced to a thiol group to form a thiol-modified oligomer;

d) said thiol-modified oligomer is purified under degassed conditions;

e) a conjugation reaction using a purified A-L and a purified thiol-oligomer is executed under degassed conditions to form an A-L-P conjugate; and

20 f) the synthesized A-L-P conjugate is purified.

53. The method of claim 52, wherein steps b)-f) are carried out using size exclusion chromatography.

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54. The method of claim 52, wherein said A-L-P conjugate is purified using electrophoresis.

55. The method of claim 52, wherein said A-L-P conjugate is purified by using high pressure liquid chromatography (HPLC).

56. The method of claim 55, wherein said HPLC is reverse phase.

57. The method of claim 52, where said disulfide-modified oligomer is purified to greater than 95% homogeneity to remove any trace of low molecular weight thiol contaminants.

10 58. The method of claim 57, where said disulfide-modified oligomer is purified to greater than 99% homogeneity.

15 59. A method for the synthesis of a radiolabeled conjugate comprising the radiolabel of an A-L-P conjugate containing an oligonucleotide or an s oligonucleotide analog; wherein

20 a) a disulfide linker is incorporated into the 5'-end and a trinucleotide tracer unit, 5'-T-3'-ps-3'-T-ps-T-5', is incorporated at the 3'-end of an oligonucleotide analog during solid-phase synthesis;

25 b) the disulfide- and tracer-containing oligomer is purified;

c) the disulfide is reduced to a thiol group to form a thiol-modified oligomer;

d) said thiol-modified oligomer is purified using size exclusion chromatography under degassed conditions to remove unreacted reagent and impurities;

25 e) a purified A-L is conjugated to a purified thiol-oligomer under degassed conditions to form an A-L-P conjugate;

f) the tracer unit is enzymatically phosphorylated to incorporate a radiolabeled phosphate into the A-L-P conjugate using PNK and radiolabeled ATP; and

g) the radioactive phosphate group of the ATP conjugate is chemically modified with an amine to protect it from cellular enzymatic degradation.

5 60. The method of claim 59, wherein the A-L-P conjugate is radiolabeled with  $^{32}\text{P}$ .

61. The method of claim 59, wherein the A-L-P conjugate is radiolabeled with  $^{35}\text{S}$ .

10 62. The method of claim 59, wherein said amine is a primary amine.

63. The method of claim 59, wherein said primary amine is ethylenediamine.

15 64. A pharmaceutical composition comprising a construct according to claim 1 and at least one pharmaceutically acceptable excipient or carrier.

65. The pharmaceutical composition of claim 64 wherein said oligomer binds to a hepatitis virus.

20 66. The pharmaceutical composition of claim 65 wherein said hepatitis virus is HDV.

25 67. The pharmaceutical composition of claim 65 wherein said hepatitis virus is HCV.

68. The pharmaceutical composition of claim 65 wherein said hepatitis virus is HBV.

*Sub A<sup>20</sup>*

69. The pharmaceutical composition of claim 68 wherein said oligomer comprises a sequence selected from the group consisting of  
<sup>5'</sup>GTTCTCCATGTCAG<sup>3'</sup>, <sup>5'</sup>TTTATAAGGGTCGATGTCCAT<sup>3'</sup>, and  
5 <sup>5'</sup>AAAGCCACCCAAGGCA<sup>3'</sup>.

70. The pharmaceutical composition of claim 68 wherein the A-L moiety of said construct is YEE(ahGalNAc)<sub>3</sub> - SMCC.

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10 71. The pharmaceutical composition of claim 70 wherein said construct is selected from the group consisting of YEE(ahGalNAc)<sub>3</sub> - SMCC -  
<sup>5'</sup>GTTCTCCATGTCAG<sup>3'</sup>, YEE(ahGalNAc)<sub>3</sub> - SMCC -  
<sup>5'</sup>TTTATAAGGGTCGATGTCCAT<sup>3'</sup>, and YEE(ahGalNAc)<sub>3</sub> - SMCC -  
<sup>5'</sup>AAAGCCACCCAAGGCA<sup>3'</sup>.

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*Add A<sup>22</sup>*